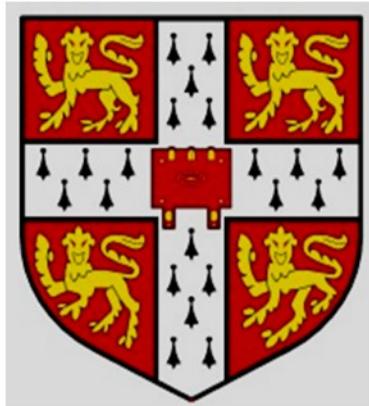


# Epigenetic disease

Imprinting disorders  
Environmental insult and disease  
associations



Claire Quilter: [craq20@cam.ac.uk](mailto:craq20@cam.ac.uk)

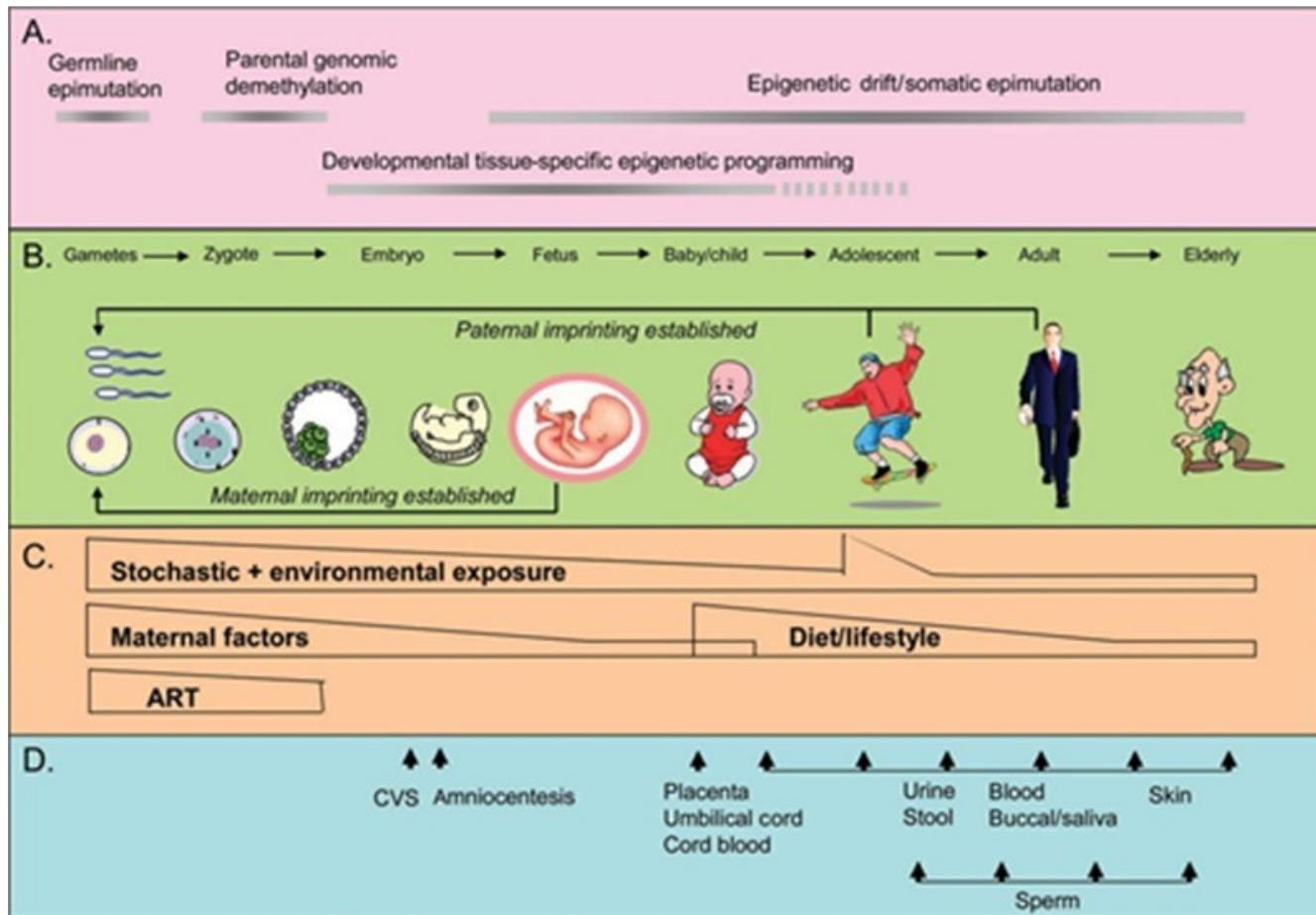
# Background

- Several systems initiate and sustain epigenetic silencing
- Disruption of one or other of these interacting systems can lead to inappropriate expression or silencing of genes, resulting in ‘epigenetic diseases’.
- Increasing number of investigations looking at the epigenetic contribution to disease

# Epigenetic signature

- An individual's behaviour, stress response, disease susceptibility and even longevity are influenced by their epigenetic signature.
- Importantly potentially malleable and thus potentially reversible, through lifestyle factors such as nutrition, exercise, behaviour modification and stress reduction.
- Such behavioural modifications have the capacity to impact complex, multifactorial diseases e.g. cardiovascular system.

# Epigenetics and disease



In Saban et al. (2014) Aging Dis; 5(5): 346–355. Epigenetics over the lifespan.

# Developmental plasticity

- Developmental plasticity is the ability of one genotype to produce a range of phenotypes in response to environmental insult.
- This can be at the level of:
  - individual cells
  - an organ
  - a whole organism

# Epigenetic disease

Abnormalities associated with:

- Embryo development
  - Hydatidiform mole
- Growth
  - BWS, SRS, pUPD14/mUPD14 syndrome
  - Cancer

# Epigenetic disease

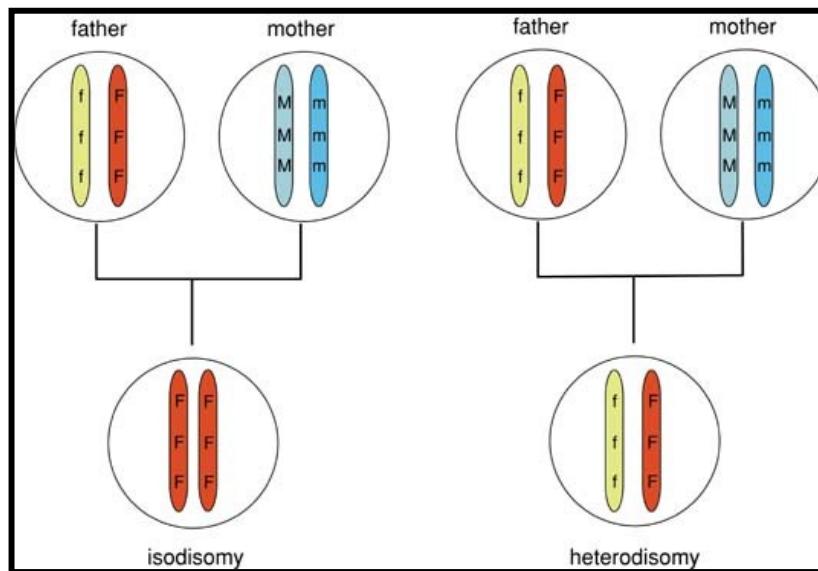
- Brain
  - Behaviour – PWS, AS
  - Psychiatric disorders
  - Neurodegenerative diseases
- Nutrient metabolism
  - Neonatal diabetes
  - Adult metabolic disease
- Endocrine system
  - Pseudohypoparathyroidism 1A, 1B.

# Genomic imprinting

- Imprinted genes represent a class of approximately 100 genes, which are expressed in a parent-of-origin specific manner.
- Essential role in normal growth and development.
- Evolved as a mechanism to balance parental resource allocation to the offspring.

# Imprinted genes – historical aspect

- Experiments in mice with UPD found P of O localized to specific chromosomes.
- Then narrowed down to gene clusters and in some cases single genes – first in mouse *Igf2r*.



~ 100 in mice, 50 humans  
<http://www.har.mrc.ac.uk/>

# Imprinting - Parent of origin

- Imprinted alleles are silenced such that the genes are either expressed **only** from the non-imprinted allele which is either paternal or maternal - epialleles.
- They are not distributed evenly but instead often form clusters.
- Epigenetic modifications which are different on each parental inherited chromosome control allele specific gene expression.

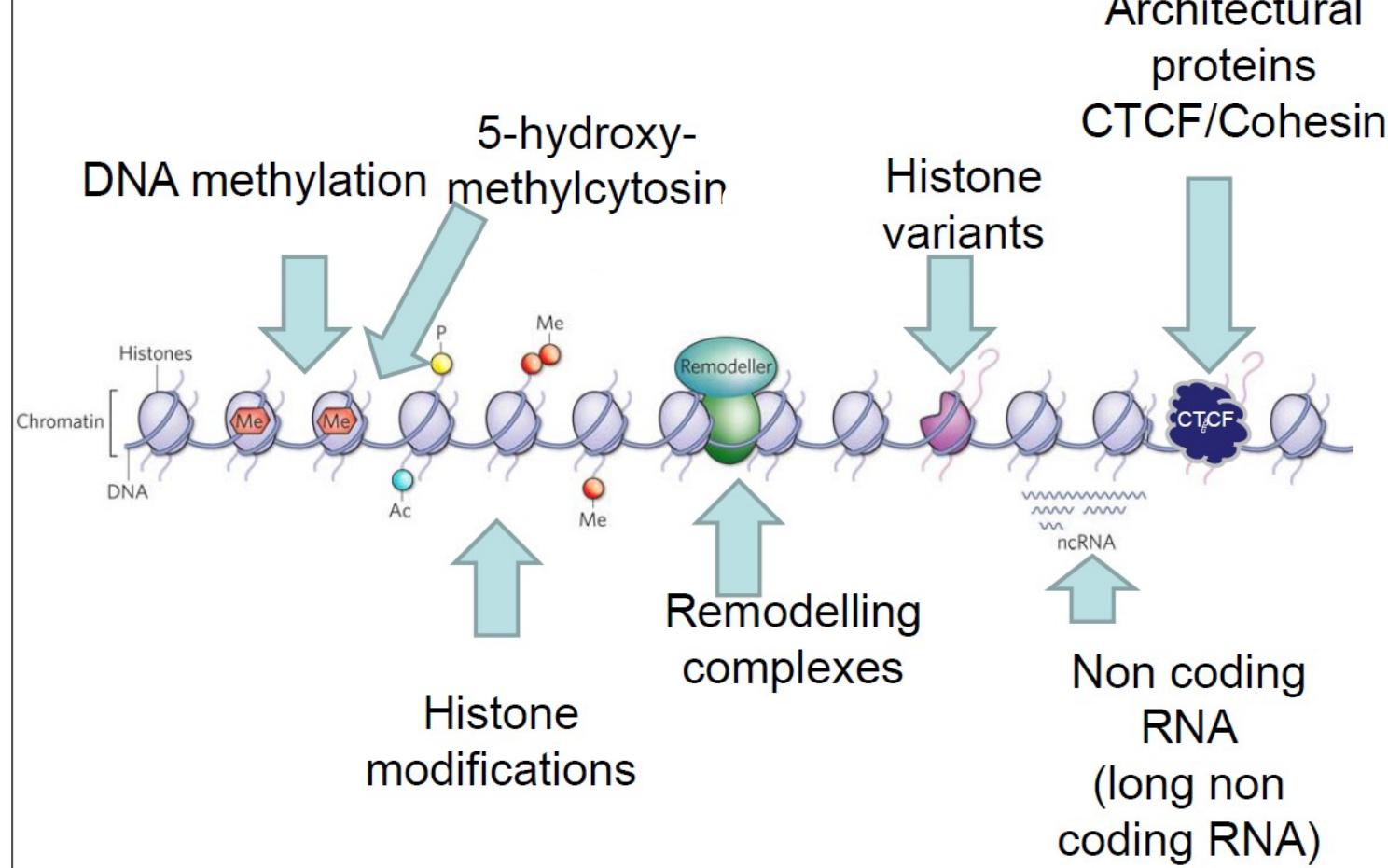
# Epigenetic mechanisms

- Predominantly differences in methylation between parental alleles – DMR (germline and somatic).
- Post –translational modification of core histones is also important.
- Under control of ICR, IC, or ICE – differentially methylated in germline.

# Epigenetic mechanisms

- Common features of imprinting clusters are:
  - Enrichment for CpG – methylation
  - Allelic histone modifications – repressive or active chromatin
  - High number of tandem repeats
  - Presence of CTCF and YY1 transcription factor binding sites
  - ncRNA transcriptional units

# Epigenetic processes provide a mechanism for environmental factors to modulate gene activity.



# Genomic imprinting

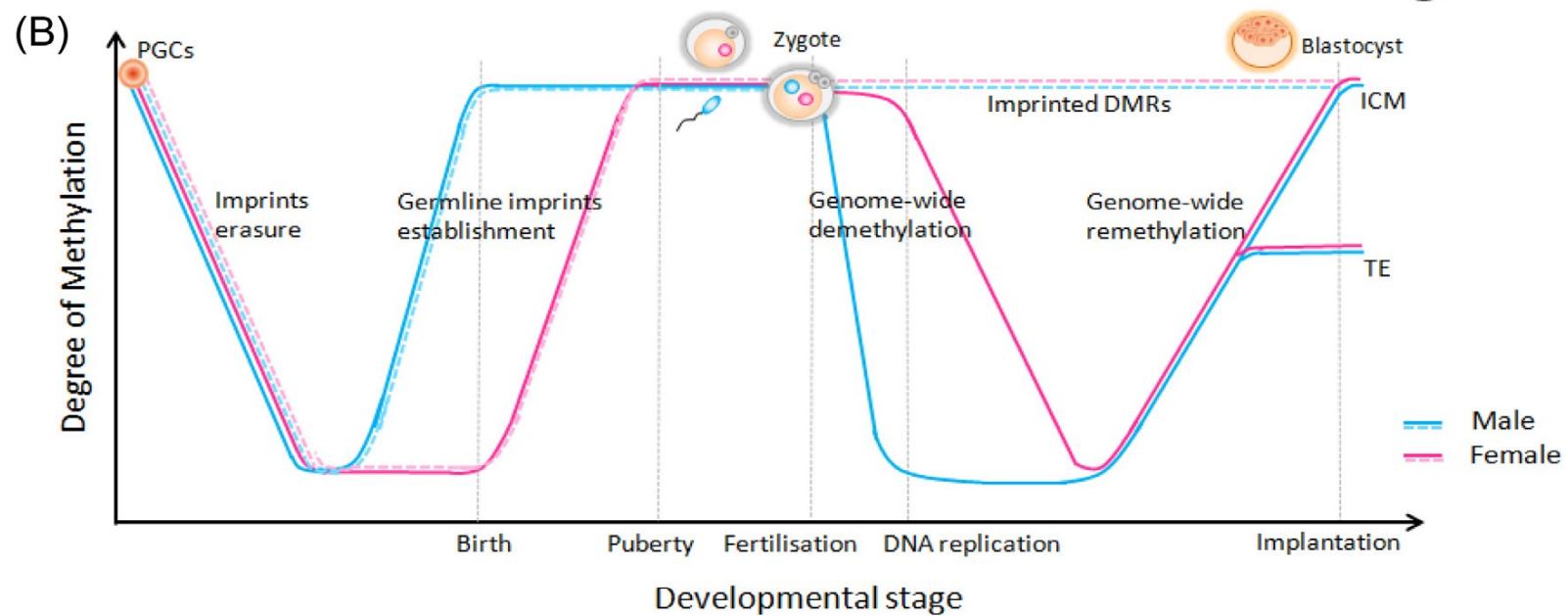
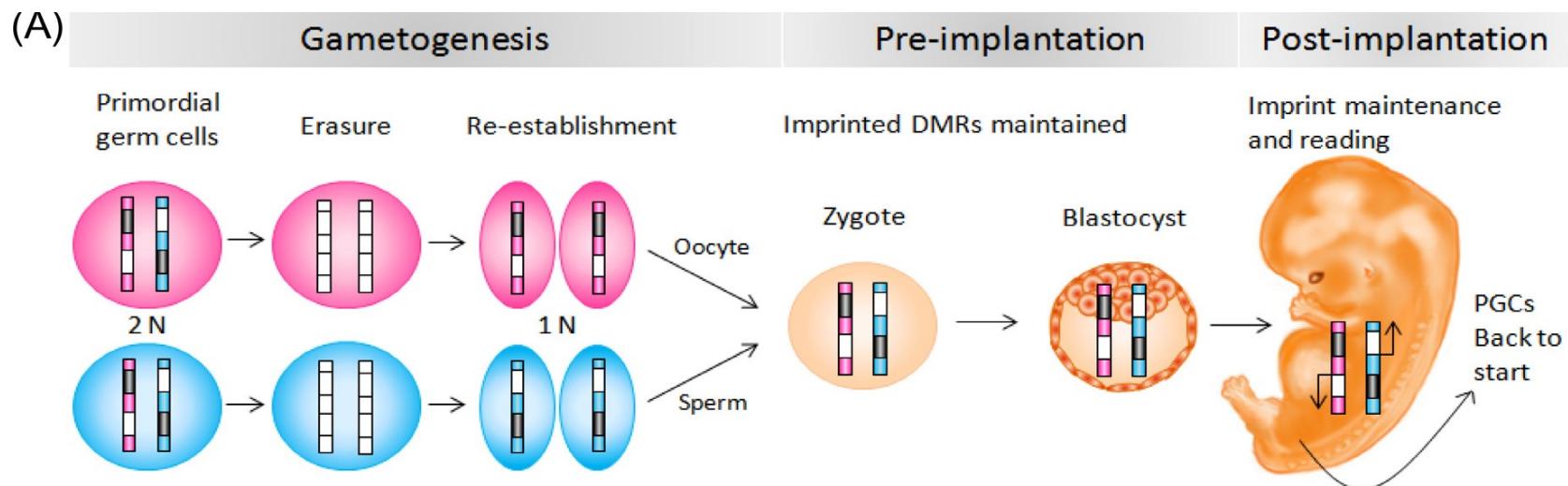
- Genetic and epigenetic disruptions which alter specific dosage of imprinted genes can result in various developmental abnormalities often associated with fetal growth and neurological behaviour.
- Methylation of the imprinted allele results in the same phenotype as when the imprinted allele is deleted – evidence that it's causing the phenotype.
- Genomic imprinting establishes the principle of transgenerational epigenetic inheritance

# Imprint erasure/establishment of methylation in the germline

- Imprinted genes are active or silent depending on epigenetic marks placed in the parental generation that survive erasure and pass on to the offspring.
- Controlled by DNMT1, DNMT3a and DNMT3b in mammals.
- DNMT3a methylates imprinted regions in conjunction with DNMT3L (non-enzymatic cofactor Dnmt3-like protein).
  - In mice KO of Dnmt3a and Dnmt3L can prevent maternal methylation.

## Imprint erasure/establishment of methylation in the germline

- How DMRs of imprinting clusters are recognised and targeted for sex specific acquisition of DNA methylation is poorly understood.
- CpGs at 8-10 bp from maternal germline DMRs.
- Histone modification – H3K4 interacts with DNMT3L.
- Transcription - open chromatin allows access to methylation of DMRs.



Ishida M, Moore GE. The role of imprinted genes in humans. Mol Aspects Med. 2013 Jul-Aug;34(4):826-40.

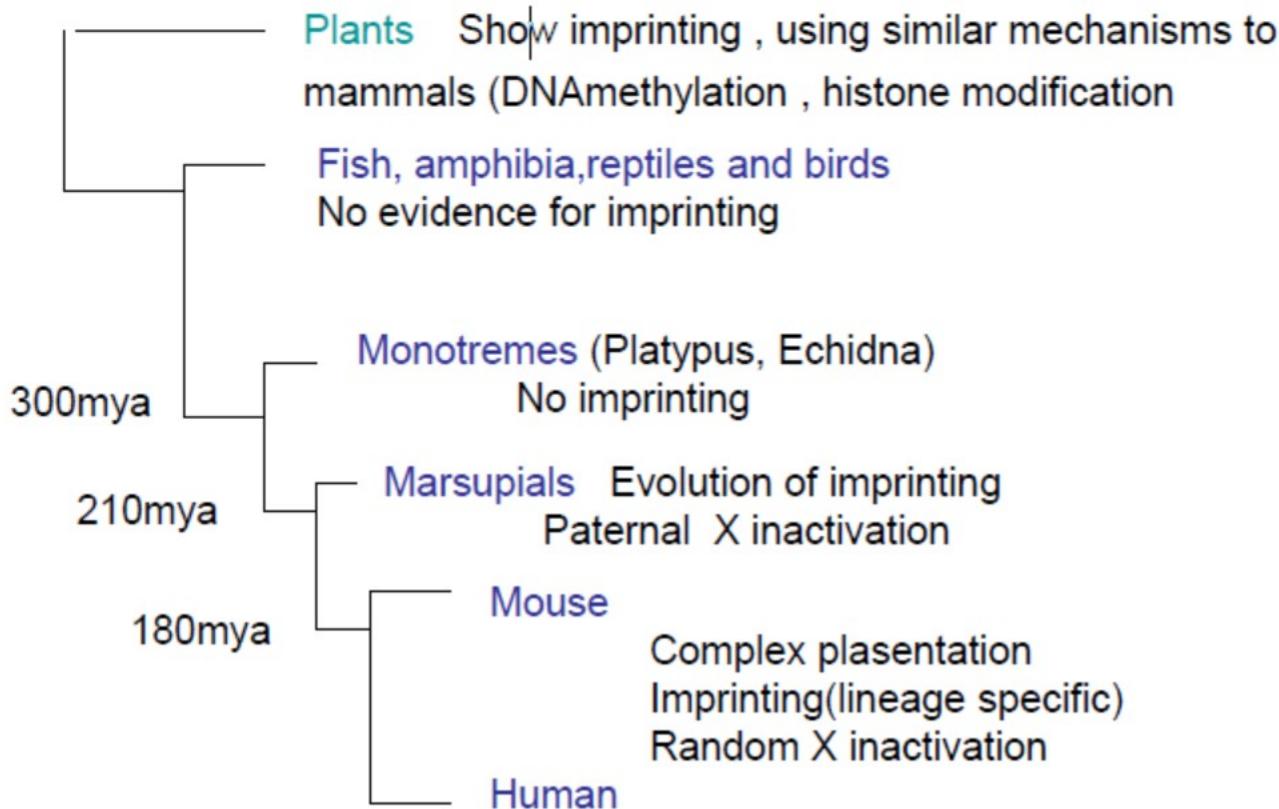
# Maintenance of imprinting

- Paternal rapid, maternal passive demethylation
- Parental specific imprint retained – ? through maternal protein DPPA3.
- Implantation – re-methylation, DNMT1 maintains methylation of imprinted loci.
- Studies in mice have shown loci that escape demethylation may be associated with repeat sequences and adjacent to transposable elements – signatures computationally.

# Evolution of imprinting

- Diploid state protects against increased exposure to deleterious mutations and or epimutations at one allele. Selection for mono-allelic expression seems paradoxical?
- Predominantly in eutherian animals (placental) but not in prototherians (egg-laying mammals) – birds and reptiles.
- To explain the emergence of imprinting – kinship theory.
  - Paternally expressed genes driven to promote fetal growth – extracting max resources from mother.
  - In contrast imprinted maternal genes are to ensure her survival and equal allocation of nutrients between offspring.
- Typically imprinting genes involved in fetal growth, placental growth, suckling and nutrient metabolism.

# Evolution of Imprinting

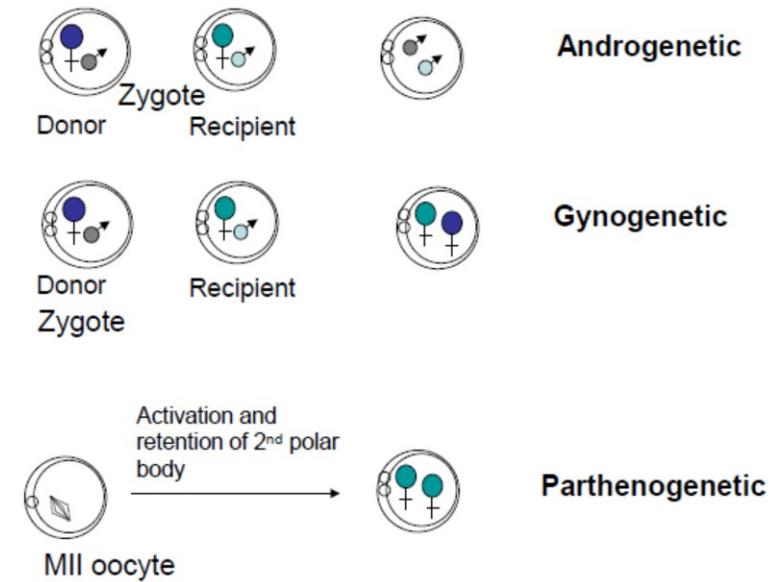


# Non - equal contribution of parental genomes?

- Parthenogenesis - maternal genes only.
- Androgenesis - paternal genes only.

# Nuclear transfer

- Fertilised egg contains 2 pronuclei – epigenetically different.
- Early evidence of P of O effects ~ 40 years ago
- 80's mouse experiments using pronuclear transplantation – diploid mouse embryos either 2 female pronuclei or 2 male pronuclei - not viable.



**Both mat and pat genomes essential for normal embryo development – not functionally equivalent.**



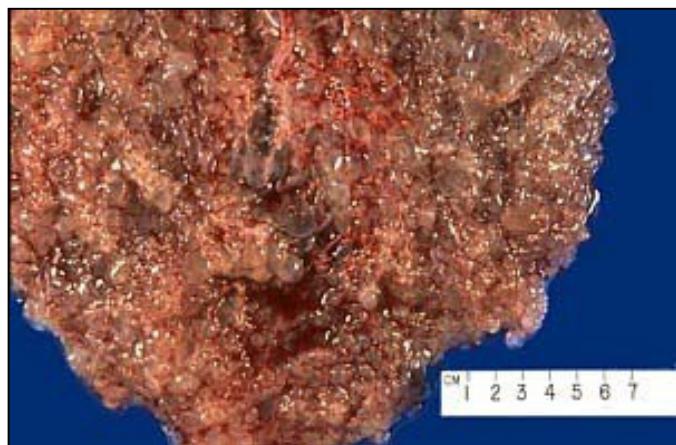
## No-Dad Dragons: Komodo's reproduce without males

Two female Komodo dragons in zoos have startled their keepers by laying viable eggs without any contribution from males. Parthenogenesis.

*'Flora is one of two zoo Komodo dragons that have laid viable eggs without mating. Inset: The fatherless child of the other solo Komodo mom works his way out of his egg'.* Chester Zoo Archives; (inset).

Buley of Chester Zoo in England and the staff at the London Zoo were surprised when, at each institution, a female with no access to males managed to have offspring. Genetic tests have verified that each female was the sole parent of her clutch, Watts et al. (2006) report *Nature*.

# Androgenetic: Hydatidiform moles



- Complete Hydatidiform moles  
Usually “empty ovum”  
fertilised by sperm which  
duplicates (46,XX) or  
dispermy 79,XXX.
- Predominantly paternal  
imprint and no maternal  
imprint.
- Rare biparental CHM – also  
predominantly paternal  
imprint – no methylation on  
maternal copies of imprinted  
genes.

# Other parental effects

- Stallion x Jenny donkey  $\text{♀}$  = hinny



- Mare x Jack donkey  $\text{♂}$  = Mule



# Human imprinting disorders

- Disruptions or epimutations of imprinted genes.
- Altered dosage of imprinted genes:
  - UPDs
  - Chromosomal duplication
  - Chromosomal deletions
- Aberrant DNA methylation of ICRs:
  - Gametogenesis – failure of erasure and re-establishment
  - Post-fertilisation – ineffective imprint maintenance

# Human imprinting disorders

- Prader-Willi, Angelman Syndrome
- Beckwith Wiedmann Syndrome
- Silver Russell Syndrome
- pUPD14/mUPD14
- Transient neonatal diabetes mellitus type 1
- Psuedohypothyroidism type 1b

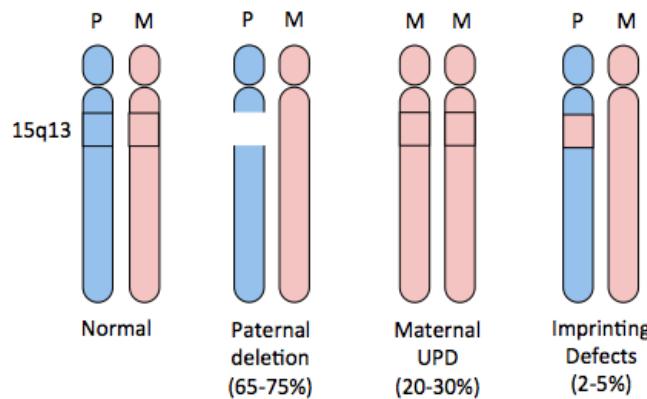
# Prader-Willi /Angelman



- Angelman and Prader Willi syndrome are rare (1 in 20K live births) neurodevelopmental disorders.
- Result from imprinting disorders in a cluster of imprinted genes at 15q11-q13.

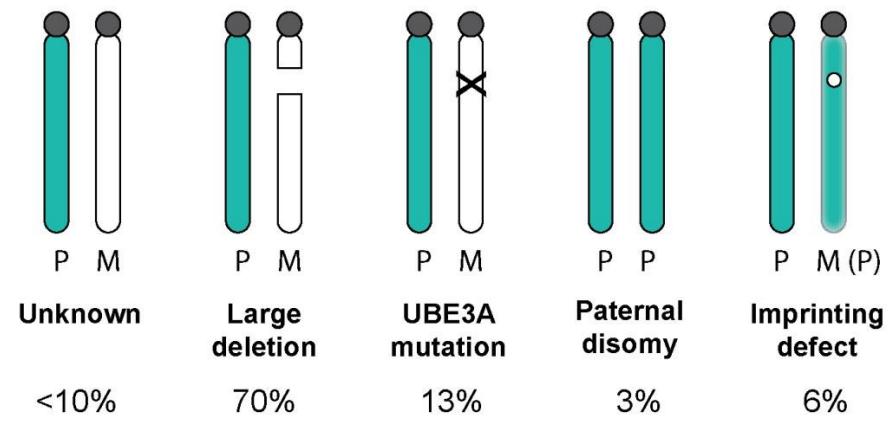


Prader-Willi syndrome : Genetic mechanisms

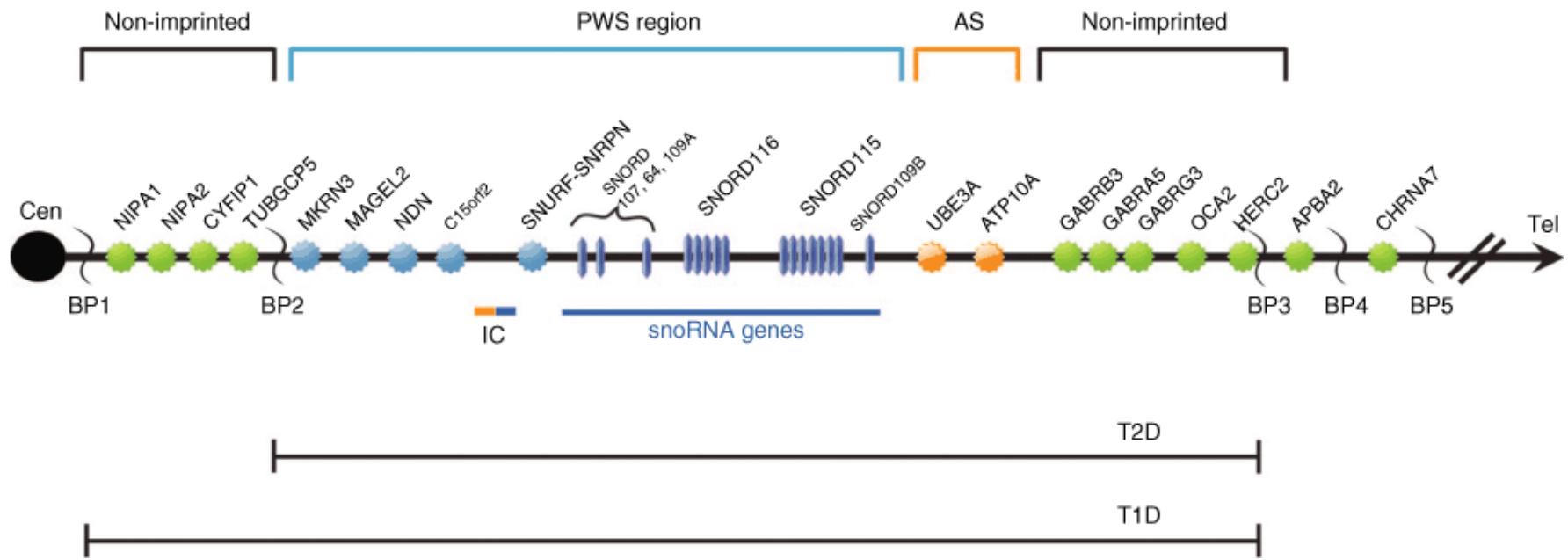


<http://www.genetics4medics.com/prader-willi-syndrome.html>

Genetic Classes of AS



# Prader-Willi Syndrome



PWS attributable to deficient expression of different genes on the pat 15 including NDN and SNORD116/HB11-85 genes which make a type of snoRNA that in addition to its standard snoRNA role, also acts to regulate alternative splicing in some target genes.

# Genetics/epigenetics of PWS

- 70% *de novo* interstitial deletion 15q11-12pat.  
Specific gene not known – SNORD116 snoRNAs  
within SNURF/SNRPN locus?
- 25% mUPD 15.
- **1-3% epimutations – DNA methylation defects through imprinted domain. PWS – ICR differentially methylated in maternal germline.**
- **Defective imprinting failure to erase grandmaternal imprint in paternal germline?**



# Prader-Willi Syndrome

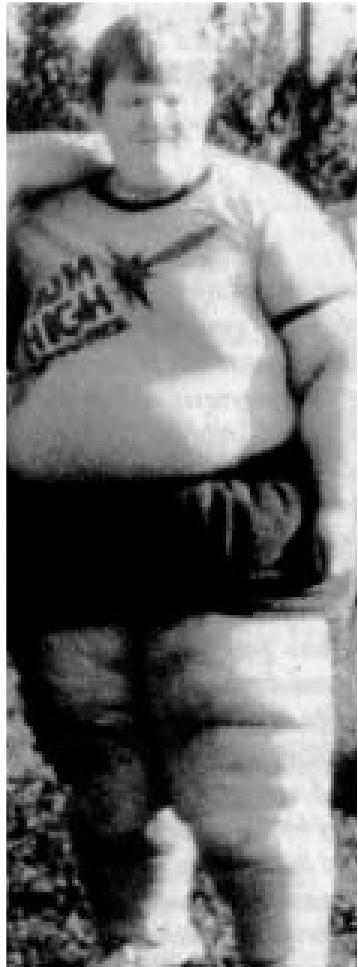
- Hypotonia: weak muscle tone, and floppiness at birth, poor suckling.

~ 1 in 17500



- Hypogonadism: immature development of sexual organs and other sexual characteristics.
- Obesity: excessive appetite and overeating (hyperphagia), and a decreased calorific requirement owing to low energy expenditure levels.

# Prader-Willi Syndrome



- Central nervous system and endocrine gland dysfunction: with varying learning disability, short stature, somnolence, and poor emotional and social development.
- Characteristic facial and other physical features: almond-shaped eyes, a narrow forehead, a down-turned mouth with a triangular-shaped upper lip, and small hands and feet.

# Genetics/Epigenetics of AS

- 70% *de novo* interstitial deletion (4-6Mb) 15q11-13mat.
- 2-3% pUPD 15.
- 10% mutation in maternally expressed *UBE3A*.
- **2-4% epimutations (paternal only methylation but biparental inheritance ~ 10% deletion of IC, rest epimutations).**
- **AS-ICR 35Kb upstream of PWS-ICR thought to establish mat imprint.**
- **Defective imprinting thought to occur during maternal imprint establishment or imprint maintenance.**



# Angelman Syndrome

- ~1 in 16000
- *UBE3A* - makes a ubiquitin protein ligase - both alleles normally expressed in most tissues but only maternal allele in neurons.
- Developmental delay, functionally severe speech impairment, none or minimal use of words; receptive and non-verbal communication skills higher than verbal ones.
- Seizures, onset usually < 3 years of age.



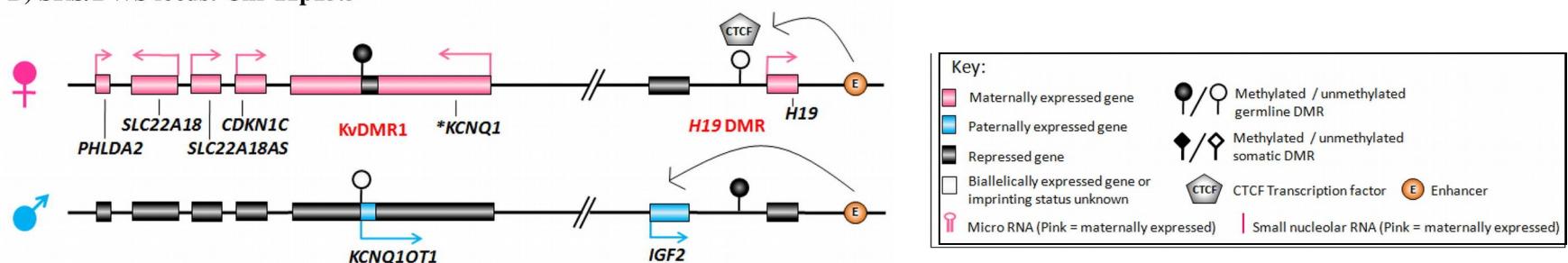
# Angelman Syndrome

- Behavioural uniqueness: frequent laughter/smiling; apparent happy demeanour; easily excitable personality, often with hand flapping movements; hypermotoric behaviour; short attention span.
- Delayed, disproportionate growth in head circumference, usually resulting in microcephaly by age 2.
- Movement or balance disorder, usually ataxia of gait and/or tremulous movement of limbs.
- Abnormal EEG, characteristic pattern with large amplitude slow-spike waves.

# Beckwith-Wiedmann Syndrome

- Two differentially methylated regions - maternal allele:
  - KvDMR1: (regulating genes *CDKN1C* and *KCNQ1*) about 50% of cases show hypomethylation – ICR of *KCNQ1* imprinting domain.
  - H19 DMR: (regulating genes *H19* and *IGF2*) 5% of cases show hypermethylation at H19 DMR - ? leading to CTCF binding inhibition and reduced *H19* and increased *IGF2* expression –

B) SRS/BWS locus: Chr 11p15.5



- 10% mutations in *CDKN1C*mat.
- 20% pUDP11.
- 15% familial of which 40% have *CDKN1C* mutation.



# Beckwith Wiedemann Syndrome

- 1 in 13,700 live births.
- Pre- and/or postnatal overgrowth - role in fetal growth – mesoderm and endoderm formation, skeletal and myogenic differentiation, also placental overgrowth.
- Features: macroglossia, umbilical hernia or exomphalos, overgrowth, hemihypertrophy, visceromegaly, hypoglycaemia, characteristic facial appearance.
- High risk for tumours – embryonal - Wilm's tumour and adrenal carcinoma.

# Silver Russell Syndrome

- 3 imprinting loci on chromosomes 7 and 11 (BWS).
- 10% mUPD 7 (possible candidates: 7q32 *MEST* and 7p12.2-3 *GRB10*). No reported epimutations to date.
- **35% - 65% hypomethylation at paternally methylated *H19* DMR 11p15.5 – dysregulation of maternally expressed *H19* and paternally expressed *IGF2* - ? by binding of CTFC to normally methylated *H19* DMR – reduced IGF2 expression and therefore growth restriction. Also hypo-, hyper- methylation at other loci (MLMD) – post fertilization imprint maintenance.**
- Maternally inherited dup 11p15 DMR1 and DMR2 (1 patient) - a SR-like phenotype.

# Silver Russell Syndrome

- Pre- and/or postnatal growth restriction – heterogenous.
- 1 in 100,000.

## Universal traits:

- Triangular face (Craniofacial disproportion)
- Down turned corners of mouth (shark mouth)
- Incurved fifth digit (clinodactyly), and usually shorter digit than normal
- Short birth length/ low birth weight
- Long, narrow head (Scaphocephaly)
- Post natal growth retardation



# Silver Russell Syndrome

## Common traits:

- Body Asymmetry
- Growth Hormone Deficiency
- Hypoglycemia
- Late closure of the fontanel
- Small chin (hypoplastic mandible) and small, crowded teeth (microdontia)
- Low set, small, or prominent ears
- Delays in bone age and muscle tone

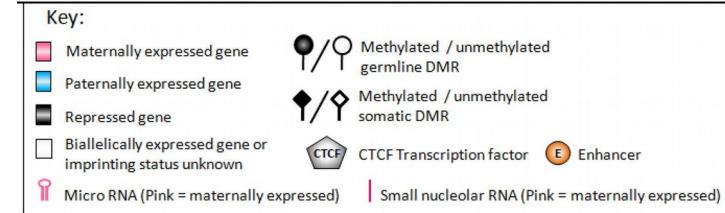
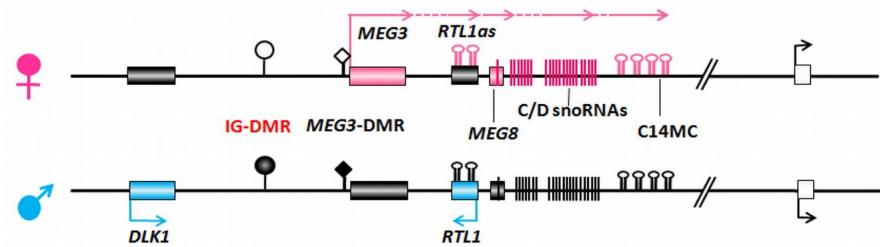


## pUPD14/mUPD14

- Deregulation of the *DLK1-MEG3* cluster 14q32.
- Both mUPD and pUPD reported epimutations and microdeletions at 14q32 on pat and mat chromosomes respectively.
- 2 paternally methylated DMRs:
  - Intergenic (IG) – DMR between *DLK1* and *MEG3* (one of few paternal germline methylated DMRs). Placental ICR.
  - Somatic *MEG3* DMR. Body ICR (methylation controlled by IG-DMR).

# pUPD14/mUPD14

C) pUPD14 /mUPD14 locus: Chr 14q32



- mUPD 14 pre- and postnatal growth restriction, premature puberty and obesity.
- pUPD 14 facial abnormality, small bell-shaped thorax, abdominal wall defects, enlarged placenta, polyhydramnios.

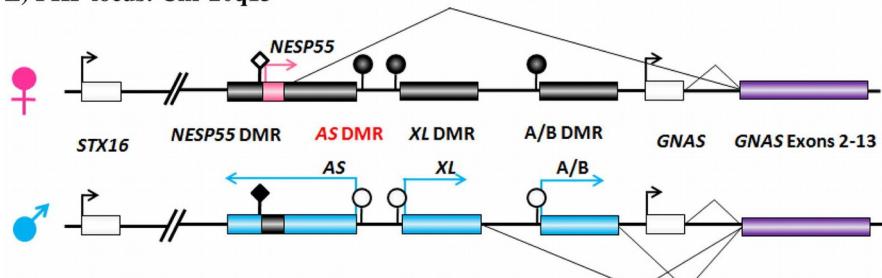
# Transient diabetes mellitus type 1

- Over-expression of paternal imprinting cluster *PLAGL1* and *HYMA1* at 6q24 – severe IUGR, hyperglycaemia (neonatal -18mths).
- 40% pUDP6.
- 32% paternal duplication.
- **28% hypomethylation at normally methylated maternal ICR – acts as promoter for the imprinted isoform of *PLAG1* – also overlaps exon 1 of *HYMA1*.**
- **~ 50% of these hypomethylated at other regions – *ZFP57* gene – maintenance of post-fertilization imprints in mice.**

# Pseudohypothyroidism type 1B

- ***GNAS1* on 20q13, hypomethylation of maternal germline allele *GNAS* exon A/B DMR and sometimes also *GNAS XL*, AS and *NESP55* DMRs.**
- **Maternal microdeletion of non-imprinted *STX16* ~220 kb upstream of exon A/B - causes hypomethylation of DMR.**

E) PHP locus: Chr 20q13



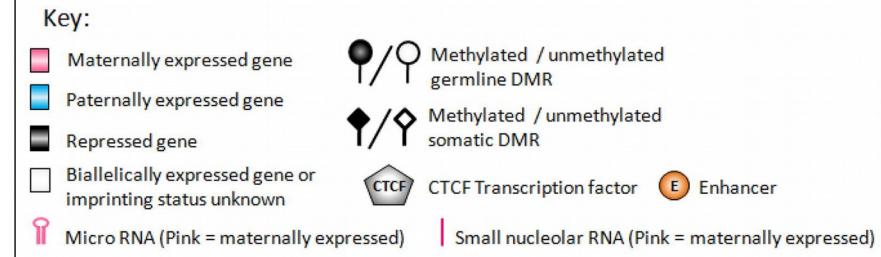
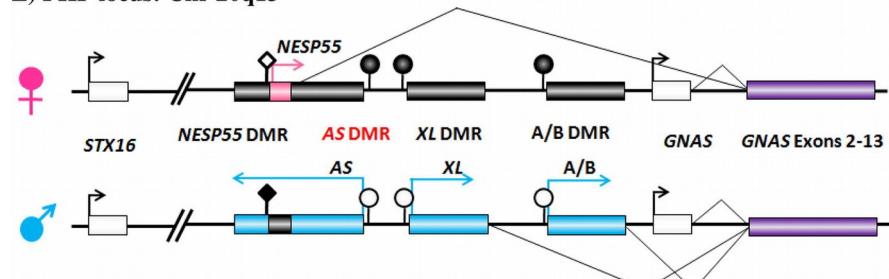
Key:

■	Maternally expressed gene
■	Paternally expressed gene
■	Repressed gene
□	Biallelically expressed gene or imprinting status unknown
●	Micro RNA (Pink = maternally expressed)
○	Small nucleolar RNA (Pink = maternally expressed)
◆/○	Methylated / unmethylated germline DMR
◆/□	Methylated / unmethylated somatic DMR
CTCF	CTCF Transcription factor
E	Enhancer

# Pseudohypothyroidism type 1B

- Maternal transmission of microdeletions overlapping exons of AS and *NESP55 DMRs* – hypomethylation
- Also hypomethylation of AS and XL DMRs suggests critical control region of whole GNAS allele in region of over-lapping deletions.

E) PHP locus: Chr 20q13



# Pseudohypothyroidism type 1B

- In mice ***Nespas*** promoter DMR is the principle ICR
- Familial and sporadic.
- Only 1 pUPD20.
- Hypoglycaemia and hyperphosphataemia due to resistance to parathyroid hormone.

# Epigenetics and disease

- Diet, stress, chemicals, pollution, drugs, smoking...
- Other disorders
  - Psychiatric
  - Metabolic – diet: obesity, diabetes
  - Cardiovascular
  - Degenerative disease
  - Cancer

# Epigenetic inheritance

- Epigenetic change may also account for high heritability's observed in numerous psychiatric diseases and apparently low identified genetic changes
- Can epigenetics account for missing heritability?
- In mice transgenerational inheritance has been shown
- Few examples of epigenetic changes across generations in humans – difficult to study.
- If the same thing happens in humans – a portion of the heritability implicated by twin studies may be partly influenced by environmental studies in the parent.

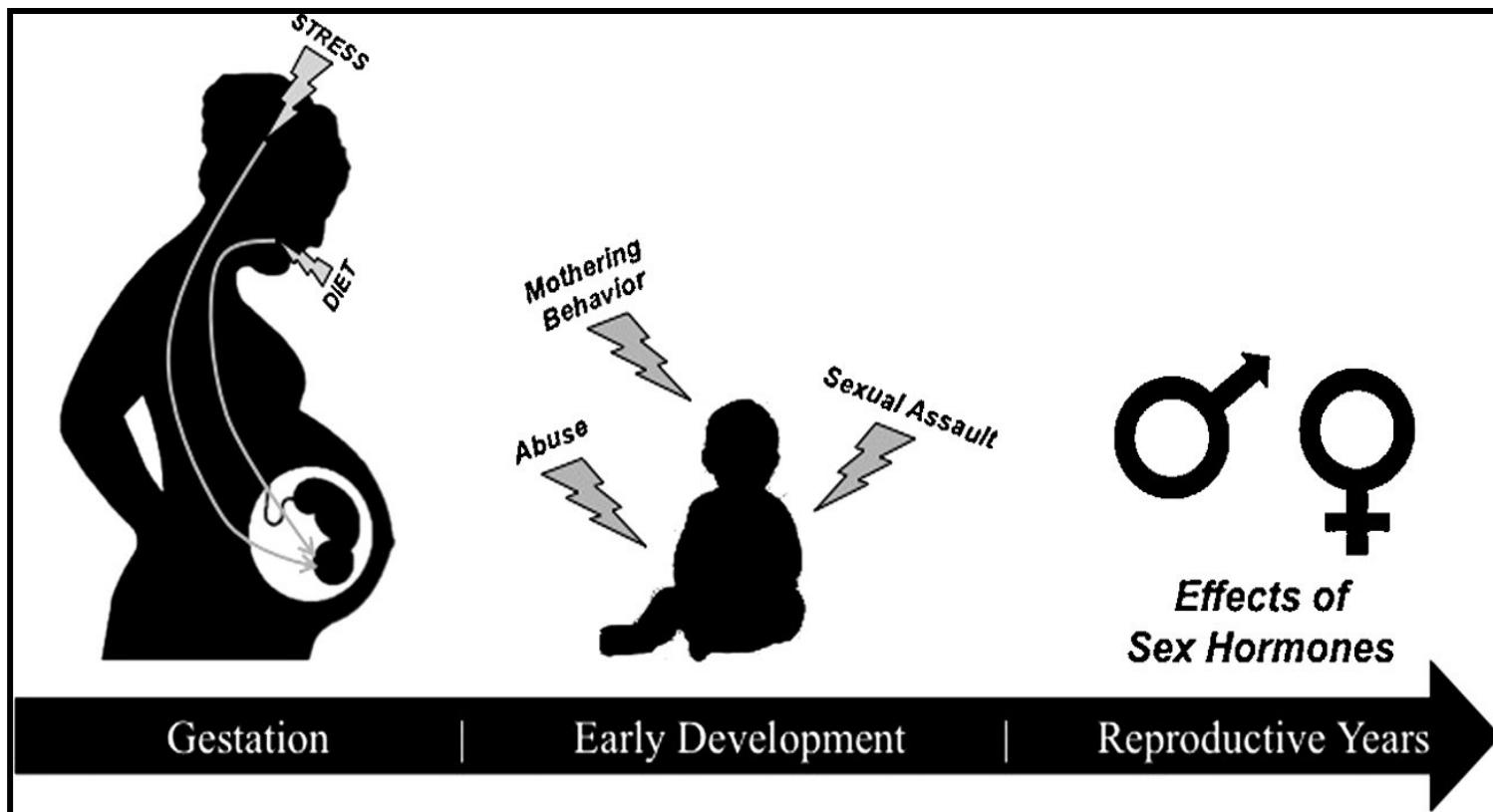
# Evidence

- Nature v Nurture - onset due to under-lying genetics with an environmental insult.
- Classical twin study design; MZ share 100% genetics, DZ share 50%.
  - Assumption twins under similar environmental influence
  - If the DZ twin group more variable for a trait compared to MZ indicates heritable factors – genetics.
  - Remainder of trait not accounted for by genetics – non-shared environmental influence? Epigenetics?
  - Limited studies on twins separated at birth – different environments.

# Heritable DNA methylation

- First study heritable component of DNA methylation (Nature 2009) MZ and DZ same sex matched twins
  - Blood
  - Buccal
- Higher DNA methylation variation between matched co twins in DZ as opposed to MZ group
- First evidence for a heritable component to DNA methylation in tissues on the genomic scale
- Confirmed with numerous studies – some degree of heritable influence on DNA methylation patterns across tissues in humans
  - Genetic
  - Non genetic/ epigenetic ? cause or effect

# Psychiatric disorders



Developmental periods sensitive to epigenetic change. An individual's risk to psychiatric disease may be modified by epigenetic changes induced by environmental insults. Depicted here are three particularly vulnerable time points: gestation, early childhood development, and reproductive years.

# Animal model

- Mice expt -neuroanatomical, behaviour and epigenetics (Dias et al 2014)
- Odour (acetophenone) paired with foot-shock
- Behavioural sensitivity to acetophenone for next 2 generations (no previous exposure)
- Structural changes in olfactory nervous system.
  - Fear conditioning and hypomethylation of *Olfr151* (activated by acetophenone) in sperm of F0 males and F1 offspring.

# Psychiatric disorders

- Twin studies shown SCZ, BP, AD, ADHD and autism - high heritability – some environmental influence but less ~ 20%.
- Major depression, anxiety, suicide, somatoform disorders and alcoholism – lower heritability –? More environmental control ~ 70%.
- Many GWAS studies – common genetic etiology across many phenotypically different psychiatric disorders – spectrum ie SCZ and BP but also SCZ and MDD and ASD.
- Conflicting genetic results in MDD –failure to account environmental influence.

# Schizophrenia

- Prenatal risk factors – SGA, mat infection, malnutrition.
- Advanced pat age, migrant status, urban, parental loss, substance abuse, hormonal changes- sex differences (environment alter HPA axis), menstruation in females (luteal).
- Mutations have been found in regions implied in epigenetic mechanism.
- Methylation differences – psychosis; in *COMT* gene, in reelin gene and dopaminergic, serotonergic, GABAergic and glutamatergic pathways – also in sperm of aging fathers.
- Histone modifications have been described, in particular the H3L4 histone methylation.

# Depression

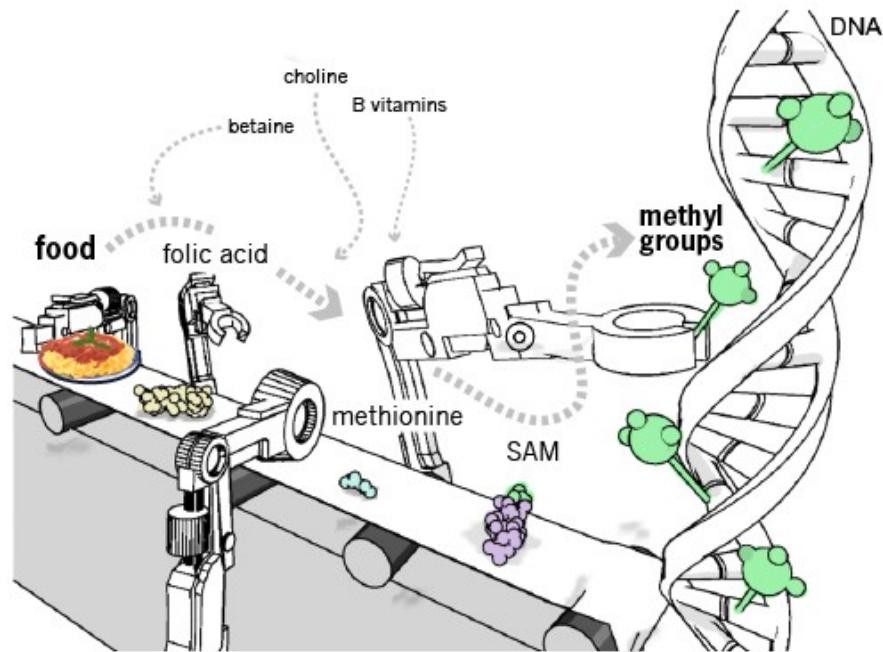
- **MDD**
  - Prenatal – high and low birth weight.
  - Early life adversity, trauma, sexual abuse hormonal fluctuation and stress, hormonal changes- sex differences (double in females after puberty).
  - DNA methylation *MORC1* in early life stress and MDD.
- **BP**
  - Caesarian section.
  - DNA methylation - brain-derived neurotrophic factor (*BDNF*)– potassium voltage- gated channel - *KCNQ3*.

# Autism Spectrum Disorder

- **ASD**
  - Prenatal - maternal (but not paternal depression) – unclear antidepressant use.
  - SGA, hormonal changes - sex differences.
  - NGS – of exome sequencing found many genes altered – incl genes involved with chromatin remodelling pathways (histone-modifying enzymes and remodelers) - most prevalent post- translational methylation/demethylation modifications of histone.
  - DNA methylation - sperm of aging fathers.

# Epigenetics and diet

- Diet is easily studied, and therefore a better understood environmental factor of epigenetic change.
- The nutrients we extract from food enter metabolic pathways where they are manipulated, modified, and moulded into molecules the body can use. One such pathway is responsible for making methyl groups.
- Familiar nutrients like folic acid, B vitamins, and SAM-e (S-adenosyl methionine, a popular over-the-counter supplement) are key components of the methyl-making pathway.



- Diets high in these methyl-donating nutrients can rapidly alter gene expression, especially during early development when the epigenome is first being established.

# Agouti mice

- Yellow Agouti A<sup>vy</sup> mouse model – coat variation correlated to epigenetic marks established in early development.
- Most widely studied murine epiallele, used to study nutrition and epigenetic impacts on the fetal epigenome.
- Wild type *Agouti* gene encodes a paracrine signalling molecule that produces either black (a allele) or yellow (A allele) coat colour or in between.
- Transient expression of A causes brown agouti mice due to yellow band on black hair shaft.

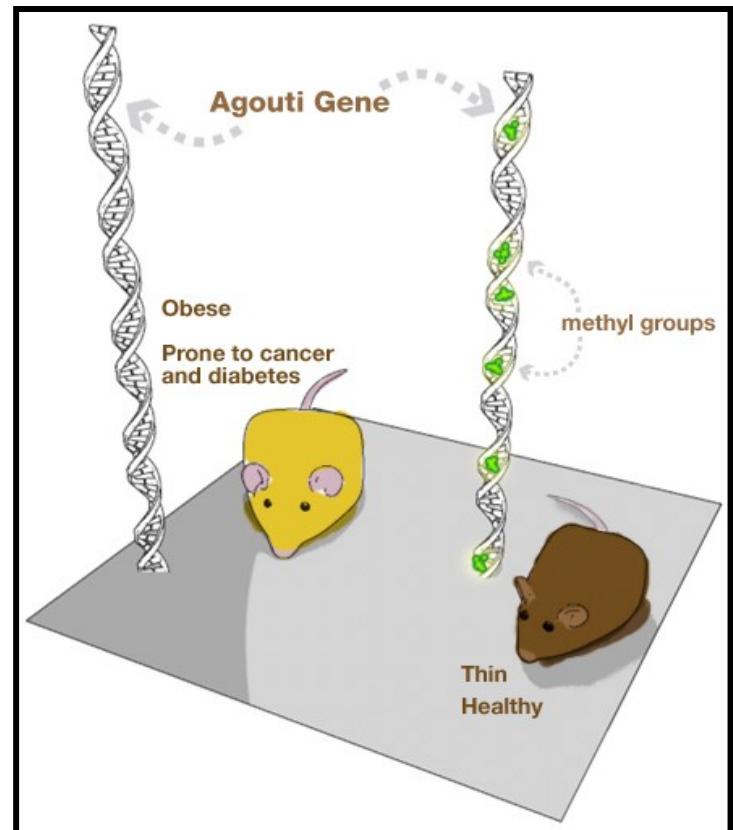
# Agouti mice

- The A<sup>vy</sup> epiallele from insertion of retrotransposon (IAP) upstream of TSS of *Agouti* gene.
- Cryptic promotor in A<sup>vy</sup> IAP promotes ectopic transcription – not just coat, all cells and leads to yellow fur and obesity, diabetes and cancer.
- Yellow mice are hypomethylated at the transposable element upstream of the *Agouti* gene allowing maximal ectopic expression.
- Yellow – unmethylated, brown – methylated (and coat colours in between)

# Agouti mice - impact of diet

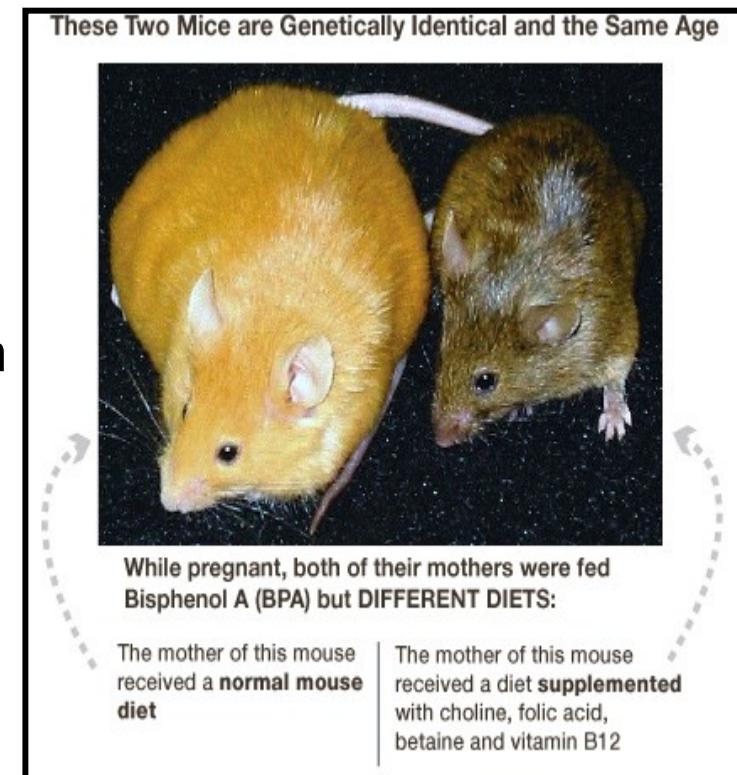


- When pregnant yellow mice were fed a methyl-rich diet, most pups were brown and stayed healthy for life.
- These results show that the environment in the womb influences adult health.
- Our health is not only determined by what we eat, but also what our parents ate.



# Agouti mice – impact of environment

- Chemicals and additives can affect the epigenome. Bisphenol A (BPA) is a compound used to make polycarbonate plastic. It is in many consumer products, including water bottles and tin cans.
- When pregnant yellow agouti mothers were fed BPA, more yellow offspring born as exposure to BPA during early development decreased methylation of the agouti gene.
- However, when BPA-exposed, pregnant yellow mice were fed methyl-rich foods, the offspring were predominantly brown. The maternal nutrient supplementation had counteracted the negative effects of exposure – added methyl groups to agouti gene.



[http://learn.genetics.utah.edu  
/content/epigenetics/nutrition/](http://learn.genetics.utah.edu/content/epigenetics/nutrition/)

# Thrifty phenotype

- Hypothesis that both high and low BW associations reflect phenotypic adaption to environmental stimuli and early nutrition – *developmental plasticity*
- Mechanism may be epigenetic which may allow favourable adaptations ie *thrifty phenotype* where get protection against under nutrition in pregnancy
- This phenotype in a richer postnatal diet ultimately may lead to metabolic disease

# Dutch famine 1944

- First data to support hypothesis that early life environment can cause epigenetic changes that persist in humans came from individuals with prenatal exposure to the Dutch Hunger Winter (1944-45)
- 6 decades later imprinted *IGF2* gene less methylation than unexposed same sex siblings
- More recently shown that women exposed to this famine had increased risk of adult type 2 diabetes

# Nutritional programming of health and disease

- Women who are pregnant, lactating, infants and children most nutritionally vulnerable
- Areas where food limited additional nutrient requirements are not met
- Undernutrition in pregnancy results in increased risk of IUGR and SGA babies
- Stunted growth
- Nutrition related factors 45% deaths <5 yrs globally

# Nutritional programming of health and disease

- In contrast to undernutrition many low to middle income countries seen an epidemic of nutrition – related chronic disease
  - Diabetes
  - CVD
  - Cancer
- Urbanisation – many who have experienced fetal or early life restriction adapt but then move to cities to a richer diet

# Peri conception

## Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia

Batbayar Khulan<sup>1,2,†,‡</sup>, Wendy N. Cooper<sup>2,†</sup>, Benjamin M. Skinner<sup>1</sup>, Julien Bauer<sup>1</sup>, Stephen Owens<sup>3,4</sup>, Andrew M. Prentice<sup>3,4</sup>, Gusztav Belteki<sup>5,6</sup>, Miguel Constancia<sup>2,7,8</sup>, David Dunger<sup>6</sup> and Nabeel A. Affara<sup>1,\*</sup>

- Our group identified the importance of periconceptional nutrition in a Gambian population, where diet is nutritionally depressed during the wet season.
- Increased death rate of individuals born in nutritionally poor seasons has been related to infection.
- Found gender-specific epigenetic changes in cord blood DNA samples, some persisted to changes in infant blood DNA.
- Significant effects also observed in postnatal samples which were not evident in cord blood.
- Identified differential methylation at genes associated with defence against infection and immune response.

# **Impact on offspring methylation patterns of maternal gestational diabetes mellitus and intrauterine growth restraint suggest common genes and pathways linked to subsequent type 2 diabetes risk**

Claire R. Quilter\*,<sup>1</sup> Wendy N. Cooper<sup>†‡§</sup>, Kerry M. Cliffe\*, Benjamin M. Skinner\*, Philippa M. Prentice<sup>‡||</sup>, LaTasha Nelson<sup>†</sup>, Julien Bauer\*, Ken K. Ong<sup>‡||</sup>, Miguel Constâncio<sup>†‡§</sup>, William L. Lowe<sup>†</sup>, Nabeel A. Affara\* and David B. Dunger<sup>‡||</sup>

# Type 2 diabetes

FASEB J. 2014 Nov;28(11):4868-79

- DNA methylation patterns using a bead chip in cord blood samples from:
  - 1) infants of mothers with GDM.
  - 2) infants with prenatal growth restraint indicated by rapid postnatal catch-up growth.
  - 3) compared with infants with normal postnatal growth.
- Differential methylation of many genes associated with diabetes, growth and development.
- Some in common to Gambian cohort and another hyperglycaemia cohort.

# Epigenetics and Cardiovascular disease

- Inflammation key in development of CHD.
- Stress triggers proinflammatory cytokines.
- Evidence - increase in proinflammatory cytokines, role mediating stress-induced vascular inflammation and atherogenesis.
- Glucocorticoids may contribute to stress-triggered endothelial dysfunction – inhibition prevention.
- Anger and endothelial dysfunction.
- Mechanisms unknown – early life, childhood, adolescence – inflammatory response, reduced cortisol response can lead to increase in CHD.
- Some evidence of DNA differential methylation.

# Epigenetics of degenerative disease

- Rheumatoid arthritis autoimmune disease characterised by chronic inflammation of the joints.
- Both genetic and environmental influences.
- Epigenetic regulation of cytokines - factors associated with inflammatory response.
  - IL-10 and IL-6 are cytokines where promoters show hypomethylation in RA patients compared to controls

# Epigenetics of degenerative disease

- Asthma is a pulmonary disorder of the airways involving several inflammatory cells and multiple mediators
- Demethylation of promoter and intronic regulatory element in IL-4 and hypermethylation of counter regulatory INF- $\gamma$  in asthmatics
- Protocadherin -20, arachidonase 12 lipoxygenase, iNOS and IL-6 are differentially methylated in asthmatics
- DNA methylated regulated genes possible biomarker targets for drug intervention
  - Lentivirus delivered short hairpin RNA suppresses the expression of IL-5 – inhibition of inflammation of airway.

# Epigenetics of degenerative disease

- Alzheimer's due to protein mis-folding of A $\beta$  peptide forming plaques.
- LINE-1 methylation increased in AD
- Age dependant epigenetic drift was also seen in MTHFR – key enzyme regulating increase in methylation of CpG islands in AD patients
- miRNA seen to be significantly expressed in AD

# Imprinted Genes and Cancer

- Dosage compensation expressed from only one parental allele.
- Maternally expressed genes – suppress growth.
- Paternally expressed genes – promote growth.
- Cancer LOH of MEGs – tumour suppressors.
- Cancer LOI of PEGs – oncogenes.

# Imprinted genes in Cancer

## Paternally Expressed

- DIRAS3
- HYMA1
- PLAGL1
- PEG10
- MEST
- IGF2
- DLK1
- KCNQ1OT1
- SLC22A18
- WT1
- AWT1
- L3MBTL
- PEG3
- NNAT

- GNASXL1
- Maternally Expressed
- CPA4
- H19
- CDKNIC
- GTL2
- NESP55

## Maternally Expressed

- CPA4
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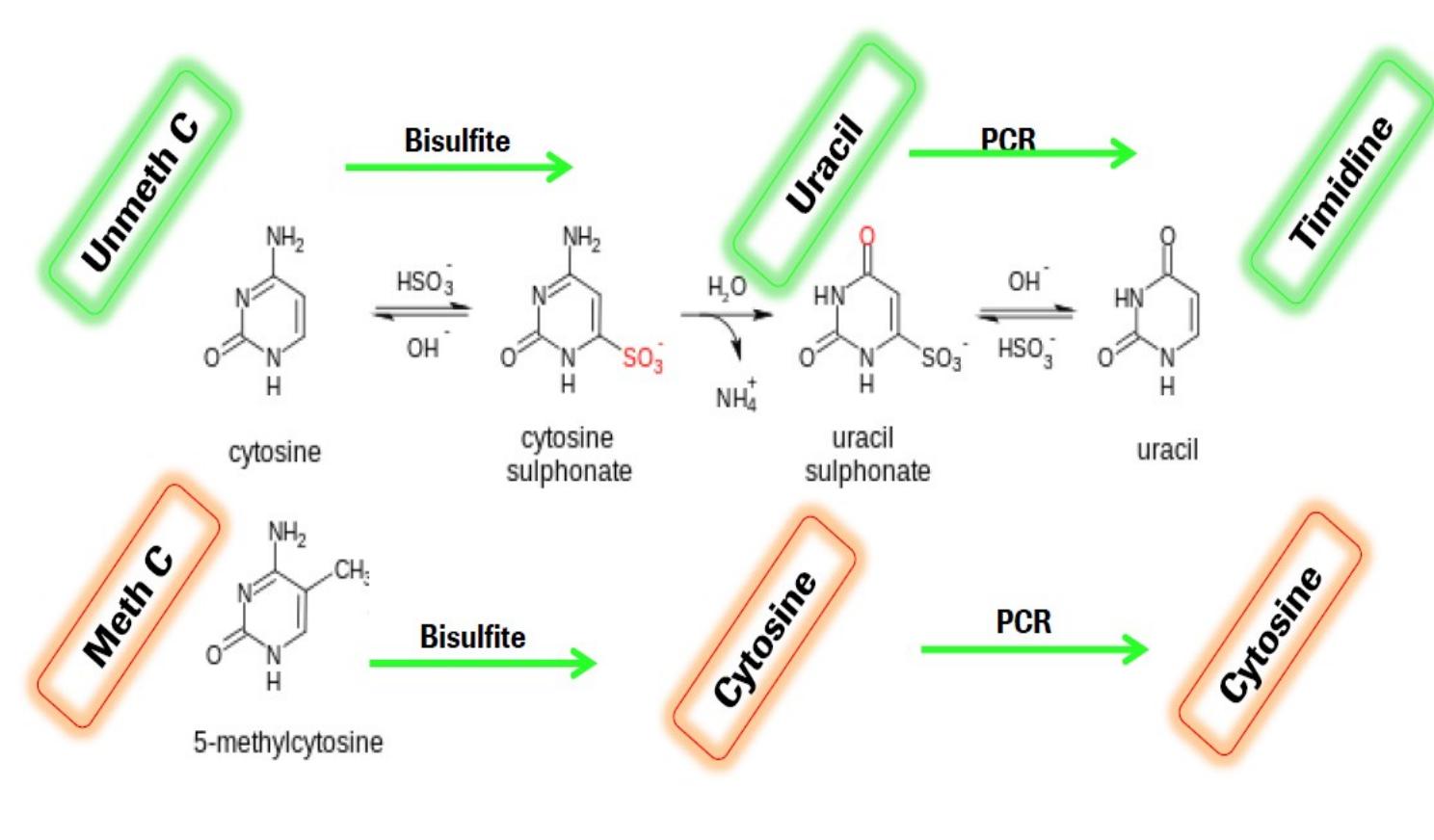
# Assisted reproductive technology

- IVF, ICSI
- Children conceived by ART ↑ risk of imprinting disorders.
- Evidence due to epimutations (Amor and Halliday, 2008).
- Embryo manipulation occurs during window of critical epigenetic reprogramming.
- Methylation defects in oocytes - cultural artefacts on methylation.
- Infertility patients may have underlying epigenetic defects.
- Sperm of infertile men have shown methylation.

# Large offspring syndrome

- Bovine and ovine embryos exposed to a variety of unusual environments prior to the blastocyst stage have resulted in the development of unusually large offspring which can also exhibit a number of organ defects.
- Four different situations have been identified that result in the syndrome:
  - *in vitro* embryo culture
  - asynchronous embryo transfer into an advanced uterine environment
  - nuclear transfer
  - maternal exposure to excessively high urea diet
- Increased incidence of difficult parturition and of fetal and neonatal losses has limited the large-scale use of *in vitro* embryo production technologies commonly used in humans and other species.

# Bisulphite conversion – common techniques for methylation studies



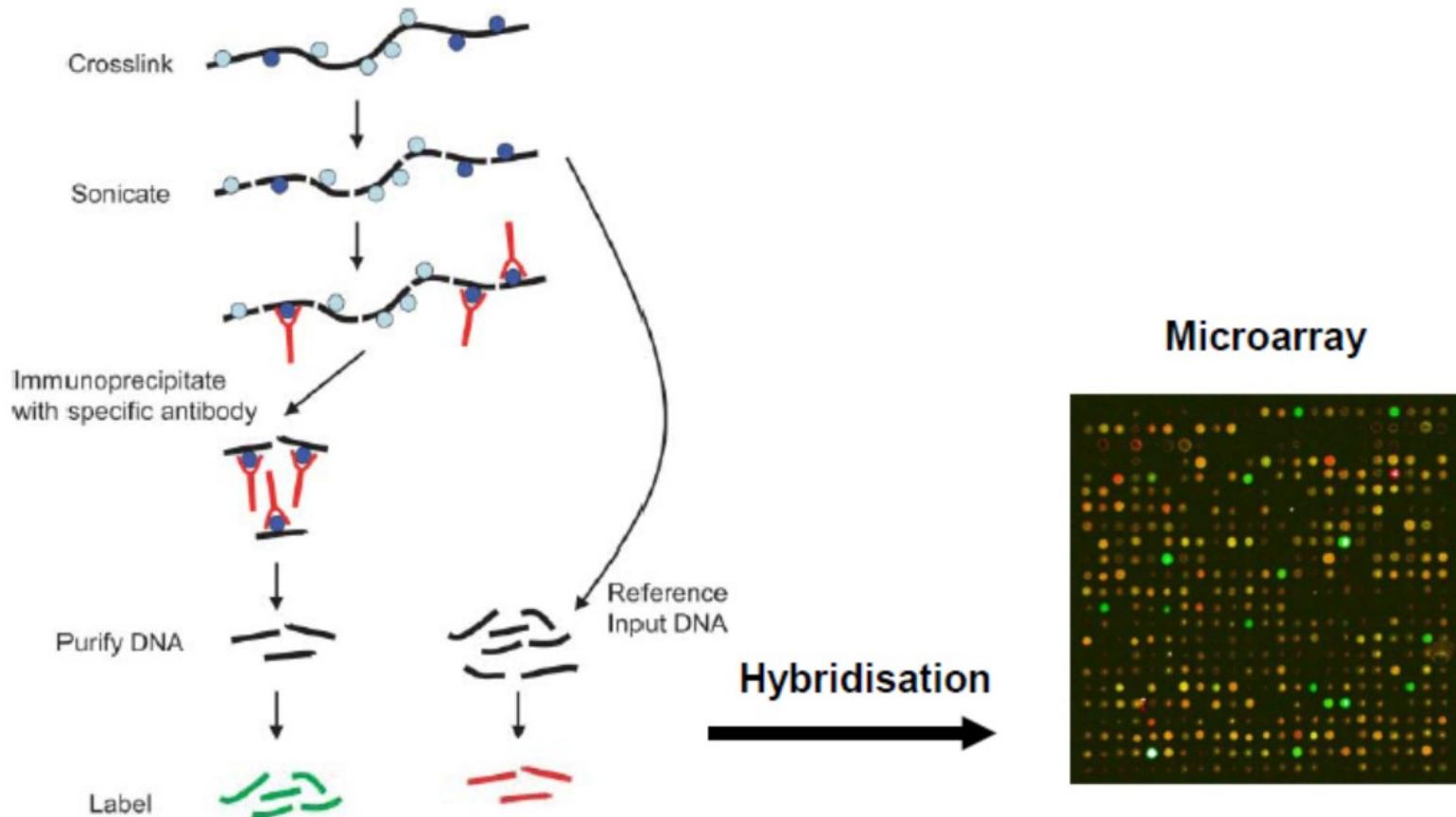
# Existing Technologies in DNA Methylation Studies

## *Limitations and gaps*

		<b>Limitations</b>
Whole Genome	<b>Whole Genome Bisulfite sequencing (WGBS)</b> 	<ul style="list-style-type: none"><li>• Poor depth of coverage</li><li>• Difficult data analysis</li><li>• Cost-prohibitive</li></ul>
	<b>Microarrays</b> 	<ul style="list-style-type: none"><li>• Indirect measurement</li><li>• Limited SNP calling on CpG</li><li>• Closed data analysis system</li></ul>
Target Enrichment	<b>Methyl-Seq</b> 	<ul style="list-style-type: none"><li>• Limited molecular complexity</li><li>• High PCR duplication</li><li>• Very high sample input</li><li>• Targets only one strand</li><li>• No custom content</li></ul>
	<b>Reduced Representation Bisulfite Sequencing (RRBS)</b> 	<ul style="list-style-type: none"><li>• Fixed content limited by enzyme sites</li><li>• Missing data</li></ul>

# Analysis of histone modifications

Chromatin Immuno Precipitation followed by microarray analysis = ChIP-on-chip



# Epigenetic therapy

- DNMTs inhibitors - clinical trials – enzymes can induce hypomethylation so inhibitors down regulate genes
- HDAC inhibitors – improved memory deficit in mice as models for AD
  - Histone deacetylases act as neuroprotectors by enhancing synaptic plasticity in AD
- siRNAs treat disease by silencing genes
- Anti-miRNA treatment in AD
- SAM – universal methyl donor treatment for RA
- Promising, most need to pass clinical trials

- Honey bees on decline since 2006.
- Colony Collapse Disorder (CCD). CCD is an unexplained phenomenon that correlates with elevated incidence of pathogens, including RNA viruses in honey bees.
- Administration of dsRNA, regardless of sequence, reduced virus infection - fed bees dsRNA in sugar solution against virus, make royal jelly – pass resistance on to next generation.
  - Therapeutic potential of ncRNAs.

